

CERTIFICATE OF MAILING

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D. Macedo

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12/15/00

**DECLARATION UNDER
37 C.F.R. §1.132**

Address to:
Assistant Commissioner for Patents
Washington, D.C. 20231

Attorney Docket	CLON-008
First Named Inventor	CHENCHIK et al.
Application Number	09/417,268
Filing Date	October 13, 1999
Group Art Unit	1655
Examiner Name	B. Forman
Title	NUCLEIC ACID ARRAYS

The Assistant Commissioner for Patents
Washington D.C., 20231

Dear Sir:

I, Alexander Munishkin, am a co-inventor of the above referenced application and an employee of Clontech Laboratories, Inc., the assignee of the above the above referenced application. A copy of my C.V. is enclosed.

I hereby declare the following:

The following assay was performed to compare the hybridization characteristics of probe spots containing a single type of probe to probes spots that contain a plurality of different probes.

I. Assay Protocol

Three oligonucleotides (80-mers) were designed for a different region of certain gene, where five different genes were tested. These oligonucleotides were spotted on a glass surface as an individual sequences as well as a mixture of all three oligonucleotides. Probes for hybridization were prepared from placenta polyA+ RNA with gene specific primers. After hybridization and washing slides were exposed to phosphoimaging plate and intensity of each spot was measured. The resultant numbers are presented in the table.

Attorney Docket No.: CLON-008
U.S. Serial No.: 09/417,268

Hybridization conditions:

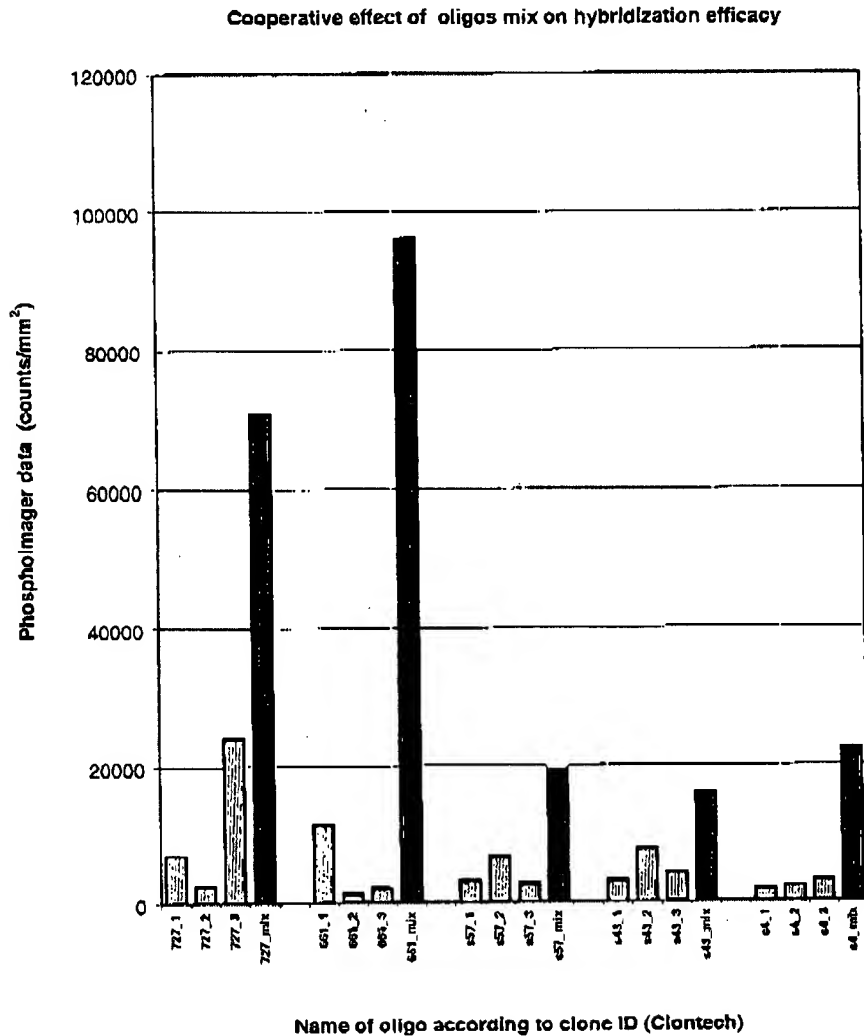
6X SSC buffer (900 mM NaCl, 90 mM Na citrate pH 7.5), 0.1% SDS

60 degree C

16 hours

V. Results

The following provides a graphical representation of the results observed from the above protocol:



VI. Discussion

The above results show that in the case where each individual oligonucleotide has very weak signal, mixture of them provides a great advantage. The effect of mixing of the oligonucleotides in one spot was not additive, rather it was cooperative. Intensity of the signal from the mixture was 2-5 times larger than the sum of the intensities from each individual oligonucleotide. This represents an unexpected result because the distance between the oligonucleotides within each probe spot on the surface is substantial and they are supposed to not interfere with each other. In other words, each oligonucleotide was expected to work independently. As such, the above observed results were unexpected.

As can be seen in the above graph, for one group of gene derived oligonucleotides probes the signal intensity of the spotted mixture was roughly the same as the sum of signal intensities of each individual oligonucleotide probe. This was in case when one of the oligonucleotides has much greater hybridization efficiency (about 5-10 times) than others.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: *December 13, 2000*Signature: *A. Munishkin*
Alexander Munishkin

enc

X C.V. of Alexander Munishkin

Alexander V. Munishkin

Education

- 1991 Ph.D. (Molecular Biology) - Moscow State University (Moscow, Russia)
- 1983 M.S. (Biochemistry) - Samara State University (Samara, Russia)
- 1981 B.S. (Biology) - Samara State University (Samara, Russia)

Professional Experience

2000 – present

Research Scientist II/ Group Leader Clontech Laboratories, Inc. (Palo Alto, CA)

1999 – 2000

Research Scientist II Clontech Laboratories, Inc. (Palo Alto, CA)

1998 – 1999

Research Scientist I Clontech Laboratories, Inc. (Palo Alto, CA).

Managing glass based microarray project, oligonucleotide based microarrays.

1995 – 1998

Research Associate (Assistant Professor). University of Chicago (Chicago, IL), Department of Biochemistry and Molecular Biology.

Research on structure of RNA molecules. Crystallization of RNA. X-ray crystallography of RNA molecules.

1992 – 1995

Research Associate (Instructor). University of Chicago (Chicago, IL), Department of Biochemistry and Molecular Biology

Identification of active center of plant toxin Ricin. Site specific mutagenesis of Ricin. Studying RNA – protein interaction. RNA mutagenesis to identify sequence essential for binding of RNA to toxin and translational elongation factor.

1991 – 1992

Research Scientist. Institute of Protein Research (Puschino, Moscow Region, Russia). Group of Biochemistry of Viral RNA.

Construction of the vector carrying recognition sites for RNA dependent RNA polymerase.

1985 – 1991

Graduate Student. Institute of Protein Research (Puschino, Moscow Region, Russia). Group of Biochemistry of Viral RNA.

Identification of recognition sites for RNA capable to replication by RNA dependent RNA polymerase from phage Q β . Creating of the library of RNA replicated by RNA dependent RNA polymerase.

1983 – 1985

Research Assistant. Institute of Protein Research (Puschino, Moscow Region, Russia). Laboratory of Regulation of Eukaryotic Translation.

Research on *Drosophila melanogaster*. Heat shock response and regulation of translation during stress treatment.

Publications.

1. Correll CC, Wool IG, **Munishkin A**. The two faces of the *Escherichia coli* 23 S rRNA Sarcin/Ricin domain: the structure at 1.11 Å resolution. (1999) *J Mol Biol* Sep 17;292(2):275-87
2. Correll CC, **Munishkin A**, Chan YL, Ren Z, Wool IG, Steitz TA. Crystal structure of the ribosomal RNA domain essential for binding elongation factors. (1998) *Proc Natl Acad Sci U S A* Nov 10;95(23):13436-41
3. **Munishkin A**, Wool IG. The ribosome-in-pieces: binding of elongation factor EF-G to oligoribonucleotides that mimic the sarcin/ricin and thiostrepton domains of 23S ribosomal RNA. (1997) *Proc Natl Acad Sci U S A* Nov 11;94(23):12280-4
4. **Munishkin A**, Wool IG. Systematic deletion analysis of ricin A-chain function. Single amino acid deletions. (1995) *J Biol Chem* Dec 22;270(51):30581-7
5. Chetverin AB, Chetverina HV, **Munishkin AV**. On the nature of spontaneous RNA synthesis by Q beta replicase. (1991) *J Mol Biol* Nov 5;222(1):3-9
6. **Munishkin AV**, Voronin LA, Ugarov VI, Bondareva LA, Chetverina HV, Chetverin AB. Efficient templates for Q beta replicase are formed by recombination from heterologous sequences. (1991) *J Mol Biol* Sep 20;221(2):463-72
7. Sitikov AS, **Munishkin AV**. [Specific suppressor T-cells secrete RNA]. [Article in Russian] (1991) *Dokl Akad Nauk SSSR*;318(6):1486-8
8. **Munishkin AV**, Voronin LA, Chetverin AB. An in vivo recombinant RNA capable of autocatalytic synthesis by Qbeta replicase. (1988) *Nature* Jun 2;333(6172):473-5

Book Chapter

Chetverin AB., Voronin LA., **Munishkin AV.**, Bondareva LA., Chetverina HV., Ugarov VI. Recombination in replicating RNAs. (1992) in "Frontiers of Bioprocessing II" (Todd P., ed.): 44-52

PhD Thesis

Structure and Origin of RNA Replicated by Q β Replicase. (1991) Moscow State University.